ORIGINAL ARTICLE

DOI: 10.4274/nkmj.galenos.2022.93063
Nam Kem Med J 2022;10(2):147-154

Measurable Residual Disease in Chronic Lymphocytic Leukemia: Experience in Real-Life Setting with Dry Tube Flow Cytometric Method

Kronik Lenfositik Lösemide Ölçülebilir Kalıntı Hastalık: Kuru Tüp Akım Sitometri Metoduyla Gerçek Yaşam Deneyimi

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ABSTRACT

Aim: Undetectable measurable residual disease (uMRD) after chemoimmunotherapy (CI) is associated longer progression-free survival (PFS) and overall survival. However, it remains to be demonstrated whether uMRD translates into survival benefit in patients treated outside of clinical trials. Pipetting-free antibody staining procedures such as dry antibody tube method can reduce process-related errors and provide a better standardization. However, there are no clinical data about this method. The aim of the study was to evaluate the impact of dry antibody tube-based MRD analysis in the management of chronic lymphocytic leukemia (CLL).

Materials and Methods: We retrospectively reviewed the data of CLL patients, who were treated with CI regimens and had MRD analysis within 6 months after therapy. Forty-six patients were included in the study. MRD was assessed by multi-color flow cytometry panels with a sensitivity level of 10^{-4}, mostly with dry tube.

Results: uMRD was achieved in 30 (65.2%) of the patients. The median PFS of patients who achieved uMRD was significantly longer compared to patients who did not. Twenty-nine patients were analyzed only by dry tube throughout study period. In the patients studied with the dry tube method, the median PFS of the ones who achieved uMRD was also significantly longer than those who did not.

Conclusion: Our study has indicated that flow cytometry based MRD surveillance of CLL patients in real-life setting provides prognostic information regarding PFS in accordance with clinical studies. In addition, clinical data of dry antibody panel (DuraClone RE CLB Tube) were presented for the first time.

Keywords: Measurable residual disease, chemoimmunotherapy, chronic lymphocytic leukemia, flow cytometry

ÖZ

Amaç: Kemoimmünoterapi (Kİ) sonrası ölçülebilir kalıntı hastalığın (ÖKH) negatifleşmesi uzun dönem progresyonsuz sağkalım (PS) ve genel sağkalım ile ilişkilidir. Ancak klinik çalışmalar dışında tedavi edilen hastalarda ÖKH negatifliğinin sağkalım üzerine etkisi belirsizdir. Kuru antikor tüp metodu gibi pipet kullanılmayan antikor boyama yöntemleri işlem ilişkili hataları azaltabilir ve daha iyi standardizasyon sağlayabilir. Fakat bu yöntemin kullanıldığı klinik veri bulunmamaktadır. Çalışmamızda kronik lenfositik lösemi (KLL) olgularının tedavisinde kuru antikor tüp metodunun etkililiğinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Kİ uygulanan ve tedavinin bitiminden sonraki 6 ayda ÖKH analizi yapılan KLL hastalarının verileri geriye dönük olarak analiz edildi. Çalışmaya 46 hasta dahil edildi. ÖKH, çoğunlukla kuru tüp metodu kullanılarak, hassasiyeti 10^{-4} olan akım sitometri ile değerlendirildi.

Bulgular: Otuz (%65,2) hastada ÖKH negatifliği sağlandı. ÖKH negatifleşenlerde medyan PS süresi negatifleşmeyenlere kıyasla daha uzundu. Çalışma sürecinde 29 hasta yalnızca kuru tüp metodu ile değerlendirildi. Kuru tüp metodu ile çalışılan hastalar ayrıca değerlendirildiğinde ÖKH negatifliğinin uzamış PS ile ilişkili olduğu görüldü.

Sonuç: Çalışmamız KLL hastalarında akım sitometri temelli ÖKH izleminin klinik çalışmalardakine benzer şekilde PS açısından prognostik önemini ortaya koydu. Ayrıca kuru antikor paneli (DuraClone RE CLB Tube) yöntemi klinik pratikte ilk kez kullanıldı.

Anahtar Kelimeler: Ölçülebilir kalıntı hastalık, kemoimmünoterapi, kronik lenfositik lösemi, akım sitometri

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Received: 24.11.2021 **Kabul tarihi/Accepted**: 07.01.2022

INTRODUCTION

In recent years, the treatment of chronic lymphocytic leukemia (CLL) has radically changed with the introduction of new biological agents[1]. However, chemoimmunotherapy (CI) agents such as fludarabine, cyclophosphamide and rituximab (FCR) and rituximab-bendamustin (RB) are still being used in first line treatment of CLL, especially in patients presenting with mutated-immunoglobuin heavy-chain variable (IGHV) or in those who do not have high-risk cytogenetic abnormalities such as del(11)(q22-23) and del(17)(p13.1)[2].

Measurable residual disease (MRD) in CLL is defined as the persistence of leukemia below the detection limit of standard assays following treatment. Undetectable MRD (uMRD) in CLL is currently defined as the presence of less than 1 CLL cell in 10,000 leukocytes ($<10^{-4}$)[3]. In CLL patients treated with CI, achieving uMRD has been found to be an independent predictor for longer progression-free survival (PFS) and overall survival (OS)[4-6]. Consequently, European Regulatory Agency (EMA) accepted the achievement of MRD negativity as an intermediate endpoint of phase III clinical trials for approval of new agents[7].

After the emergence of the new biological agents such as ibrutinib, which provide long term disease control in CLL patients but, surprisingly with persistent detectable disease[8], it seems that MRD evaluation might not have the same predictive value in patients receiving CI. However, after the inclusion of venetoclax to treatment armamentarium of CLL and novel chemo-free combination strategies started being evaluated in clinical trials, MRD evaluation attracted the attention of scientific community again[9,10].

MRD status can be evaluated via multicolor flow cytometry (FCM), real-time quantitative polymerase chain reaction (RQ-PCR) and high-throughput sequencing (HTS). Even though RQ-PCR and HTS are more sensitive methods, FCM is undoubtedly the most widely applied procedure because of the recent standardization, and the general applicability[11]. In the beginning, residual disease was assessed with 2- or 3- color FCM with low sensitivity, then the definition and applicability of MRD has dramatically changed. European Research Initiative on CLL (ERIC) in collaboration with US and Australian centers has proposed several standard assays regarding MRD evaluation with FCM, which are also recognized by regulatory agencies[11-14].

It has been claimed that the use of pipetting-free antibody staining procedures, such as dry antibody cocktails which reduce the influence of pipetting-originating errors, may result in better standardization of detection[15]. Recently, an eight-color tube with dried reagent, which is specific for detection of MRD in CLL samples, was developed by Beckman Coulter. This new technology provides tubes that contain a dry antibody panel coating adhered to the bottom of the tube[16]. This panel was compared to the method purposed by ERIC group, which uses liquid reagents, and it had been shown that the analysis of MRD in CLL samples was sensitive and feasible with this new method[16]. However, no clinical data about this method have been published until now.

Although the prognostic significance of uMRD has been demonstrated in prospective clinical trials, it is still unknown whether uMRD translates into a real benefit in patients treated off-study. Accordingly, the aim of our study was to investigate whether the end-of-treatment MRD would contribute to the management of CLL patients in real life setting. In addition, we aimed to demonstrate the validity of the dry tube method in clinical patient samples.

MATERIALS AND METHODS

Study Design

We retrospectively reviewed data of CLL patients who were treated with CI regimens [RFC, RB and R-Clorambucil (R-CLB)] at our department between June 2013 and January 2021. We included all consecutive CLL patients (aged ≥18 years) who were diagnosed as CLL according to the International Workshop on CLL (iwCLL)[17] and had received at least four cycles of CI as first line therapy. Patients were only included if data for clinical follow-up were available, and if they had complete response (CR), CR incomplete recovery (CRi) or partial response without absolute lymphocytosis according iwCLL criteria at the end of therapy and had MRD analysis performed within 6 months after therapy.

Baseline characteristics of the patients were recorded at the time of starting CI. This included age, gender, Rai stage, the indication of treatment, the interval between diagnosis and the start of CI, complete blood count values, beta-2 microglobulin, albumin, creatinine, lactate dehydrogenase, and genetic features like del(13q14), trisomy 12, del(17p), del(11q). Moreover, the cycle's number of CI, response to treatment, remission duration and other time parameters were recorded.

MRD Assessment

Fresh peripheral blood samples were used for all MRD analyses with FCM. Until March 2015, four-color two tubes FCM method with antibody panel including anti-CD19, anti-CD20, anti-CD5, anti-CD43, anti-CD81, anti-CD79b, anti-CD3 was used on a FACS Calibur (BD Biosciences, USA). Between March 2015 and February 2017, eight colors FCM, with one tube, antibody panel including anti-CD19, anti-CD20, anti-CD5, anti-CD43, anti-CD81, anti-CD79b, anti-CD3, anti-CD45 was used on a BC Navious FCM (Beckman Coulter, FL, USA). Both methods were in line with CLL ERIC recommendations[13].

After February 2017, we used eight-color DuraClone RE CLB Tube includinganti-CD81, anti-ROR1, anti-CD79b, anti-CD19, anti-CD5, anti-CD43, anti-CD20, and anti-CD45, which was in line with the latest ERIC recommendations[11]. The study procedure was performed according to the instructions of the manufacturer. Briefly, 300 micL of whole blood was added directly to the dried reagent tube and incubated in dark at room temperature for 15 minutes. Then, samples were lysed with VersaLysing Solution (Beckman Coulter) for 20 minutes, centrifuged and washed once with phosphate buffered saline (PBS). They were re-suspended in 500 micL PBS. After that, the samples were acquired in a Navios Flow Cytometer (Beckman Coulter, FL, USA). The setting and compensation matrix for samples were performed using the Compensation Kit provided in the kit according to the manufacturer's instructions. Data analysis was performed on Kaluza Flow Cytometry Analysis Software (Beckman Coulter, USA). Irrespective of the methodology used regarding flow cytometric MRD evaluation through study period, we analyzed at least 500000 cells in every case to reach a sensitivity level below 10^{-4} as recommended by ERIC. All participants gave written informed consent for the use of clinical/laboratory data for research purposes. The study was approved by Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (date: 29/09/2020 protocol number: 2020.219.09.06) and conducted in accordance with Helsinki's declaration.

Statistical Analysis

All statistical analyses were performed using the IBM Statistical Package for the Social Sciences Statistics (version 25.0; IBM Corp., USA). A descriptive analysis of continuous and qualitative variables was performed. PFS was defined as the time from the start of treatment until disease progression. Survival curves were calculated using the Kaplan-Meier method and cross-group comparisons were made using the log rank test. Univariable and multivariable analyses for association between pretreatment characteristics and PFS were performed using the Cox regression analysis. Multivariable analysis for MRD status (binary outcome) was performed using logistic regression analysis. Clinical and biological characteristics between groups were analyzed with the χ^2 test or Kruskal-Wallis test. All p-values were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

Forty-six patients were included in the study. The numbers of the patients in the FCR, RB and R-CLB groups were 24, 17 and 5, respectively. The patients' characteristics and outcomes are presented in Table 1. As expected, there was a difference in terms of age between study arms. FCR group included younger patients with normal renal function compared to RB and R-CLB arms. The same trend was observed in serum β-2-microglobulin which might be explained with differences of serum creatinine levels among study cohort. In total, results of cytogenetic assessment were available for only 22 patients (47.8%). None of the patients had del (17p). Since IGHV mutation status and ZAP 70 expression were not studied in most patients, they were not included in the analysis.

Twenty-nine patients were analyzed only by dry tube throughout the study period. Seventeen patients were analyzed previously by means of other methods but after February 2017, the analyses were repeated with dry tube method in all these cases. All the non-dry tube method evaluated positive MRD patients were found to be positive again with dry tube method. At the end of therapy, uMRD was achieved in 30 (65.2%) of the patients. Eighteen patients in the FCR group and 12 patients in the bendamustine/rituximab (BR) group achieved uMRD; however, none of the patients in the R-CLB group achieved uMRD (Table 1). Pretreatment characteristics including age, stage, CD38 expression and β-2 microglobulin were not associated with the end of treatment uMRD in multivariable analysis (data not shown).

When all cases were evaluated together, the estimated median PFS was 56 months (Figure 1A). The median follow-up time was 34.5 months (9-100 months). Only one of 46 patients died within the follow-up period. The median PFS of patients who achieved uMRD was significantly longer (not reached) compared to patients who did not (29 months) (Figure 1B). In the treatment groups, age, β-2 microglobulin, albumin and creatinine were associated with PFS in univariate analysis (Table 2) but only albumin was associated with PFS in multivariate analysis (p: 0.008, hazard ratio: 15.54, confidence interval: 2,030-119.1). Relapse occurred in only 4 of the patients who achieved uMRD, 2 of them were in the RB group, and two in the RFC group. Moreover, 13 patients who were unable to achieve uMRD relapsed.

The patients studied with the dry tube method were also evaluated separately. The median follow-up period in these patients was 25 months. Other characteristics of these patients were given in Table 3. The median PFS of the patients who achieved uMRD was significantly longer (not reached) than those who did not (22 months) (Figure 2).

DISCUSSION

Our study, which included CLL patients who were treated in real-life setting, has indicated that achievement of uMRD at the end of first line treatment of CLL with CI is significantly associated with longer PFS.

Firstly, the FCR300 and the CLL8 studies indicated the efficacy of FCR as front-line therapy for CLL[18,19]. Aforementioned studies

clearly showed that a subset of patients who had mutated IgHV and did not have high-risk cytogenetic abnormalities, such as del(17p13.1) and del(11q22.3), achieved long-term durable remissions with FCR. Whilst FCR is known to result in longer PFS compared to RB in younger patients, the benefit was not seen in patients over 65 years of age and a lower rate of serious infections were observed in the BR cohort[20]. Therefore, for older CLL patients with standard risk over the

Table 1. Characteristics and outcome of study cohort

Variable*	All patients, n=46	RFC group, n=24	RB group, n=17	R-CLB group, n=5	p value
Age (years)	65 (45-83)	58.5 (45-68)	68 (60-83)	76 (75-77)	<0.0001
Sex, n (%)					
Male	27 (58.7)	14 (58.3)	10 (58.8)	3 (60)	0.998
Female	19 (41.3)	10 (41.7)	7 (41.2)	2 (40)	
RAI stage; n, (%)					
0-2	24 (52.2)	13 (54.2)	8 (47.1)	3 (60)	0.844
3,4	22 (47.8)	11 (45.8)	9 (52.9)	2 (40)	
Indication of treatment** n, %					
1	20 (43.5)	10 (41.7)	7 (41.2)	3 (60)	0.602
2	8 (17.4)	5 (20.8)	3 (17.6)	0 (0)	
3	8 (17.4)	5 (20.8)	3 (17.6)	0 (0)	
4	2 (4.3)	0 (0)	1 (5.9)	1 (20)	
5	8 (17.4)	4 (16.7)	3 (17.6)	1 (20)	
Time from diagnosis to treatment, months, median, (range)	12 (1-156)	7 (1-48)	23 (1-81)	18 (8-156)	0.108
Cycles number, n (%)					
4	3 (6.5)	2 (8.3)	1 (5.9)	0 (0)	0.79
5	6 (13)	4 (16.7)	2 (11.8)	0 (0)	
6	37 (80.4)	18 (75)	14 (82.4)	5 (100)	
Hemoglobin, (gr/dL)	11.8 (8.2-16.26)	11.72 (8.3-14.4)	11.84 (8.2-14.4)	11.66 (10-16.26)	0.974
Lymphocyte count, (10^9/L)	50.35 (5.2-138.8)	47.3 (9.6-138.8)	59.3 (5.83-126.2)	43.97 (12.9-61.5)	0.262
Platelet count, (10^9/L)	149.5 (23.0-505.0)	180.5 (84.0-505.0)	139.0 (23.0-261.0)	120.5 (91.0-257.0)	0.291
B2M, (mg/L)	4.4 (2.1-8.0)	3.15 (2.1-6.4)	4.7 (2.6-8.0)	6.6 (3.5-7.1)	0.002
Creatinine, (mg/dL)	0.91 (0.5-1.6)	0.77 (0.5-1.31)	1.08 (0.63-1.6)	1.08 (0.55-1.49)	0.016
Albumin, (gr/dL)	4.47 (2.30-5.23)	4.35 (3.0-4.9)	4.6 (4.0-5.23)	4.7 (4.4-5.1)	0.232
LDH (U/L)	249 (120-568)	235 (127-475)	248 (178-568)	251 (232-407)	0.423
CD38 positive (>%30), n (%)	5 (11.4)	4 (16.6)	0 (0)	1 (20)	0.197
FISH (available: 22), n (%)					-
Del (17p)	0 (0)	0 (0)	0 (0)	0 (0)	
Del (11q)	1 (4.54)	0 (0)	1 (8.33)	0 (0)	
Del (13q)	7 (31.8)	2 (25)	5 (41.6)	0 (0)	
Trisomy 12	2 (9.0)	0 (0)	2 (16.6)	0 (0)	
No aberrations	11 (50)	6 (75)	4 (33.3)	1 (100)	
Responses, n (%)					
CR	31 (67.4)	16 (66.7)	12 (70.6)	3 (60)	0.22
CRi	10 (21.7)	6 (25)	4 (23.5)	0 (0)	
PR	5 (10.9)	2 (8.3)	1 (5.9)	2 (40)	
uMRD patients, n (%)	30 (65.2)	18 (75)	12 (70.6)	0 (0)	0.005
Relapsed patients, n (%)	17 (37.0)	7 (29.2)	7 (41.2)	3 (60)	0.388

*Parametric variables are expressed as median (minimum-maximum).

**1: Evidence of progressive bone marrow failure 2: Massive/symptomatic or progressive splenomegaly/lymphadenomegaly 3: progressive lymphocytosis with an increase of >50% over a 2-month period or lymphocyte doubling time of <6 months. 4: Autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids 5: Constitutional symptoms.

B2M: β-2 microglobulin, LDH: Lactate dehydrogenase, FISH: Fluorescence *in situ* hybridization, CR: Complete response, PR: Partial response, CRi: CR with incomplete bone marrow recovery, uMRD: Undetectable measurable residual disease, RFC: Rituximab, fludarabine, cyclophosphamide, RB: Rituximab, bendamustine, R-CLB: Rituximab, clorambucil

age of 65 years, BR is a logical first line treatment option with a good risk-benefit ratio, unless there is a contraindication to bendamustine. In this case, chlorambucil plus rituximab could be used and preferred over BR[20]. The CLL11 trial evaluated the impact of obinituzumab in older patients with co-morbidities[21]. Treatment with obinutuzumab-CLB compared with R-CLB and CLB monotherapy increased response rates and prolonged PFS. Finally, although the role of CI in CLL treatment has narrowed with the introduction of venetoclax and BTK inhibitors into CLL treatment, CI is still used especially in CLL patients with good prognostic features[2]. As we have mainly treated CLL patients with CI until recently, CLL patients who were followed and treated with CI between the years of 2013 and 2020 were included in our study.

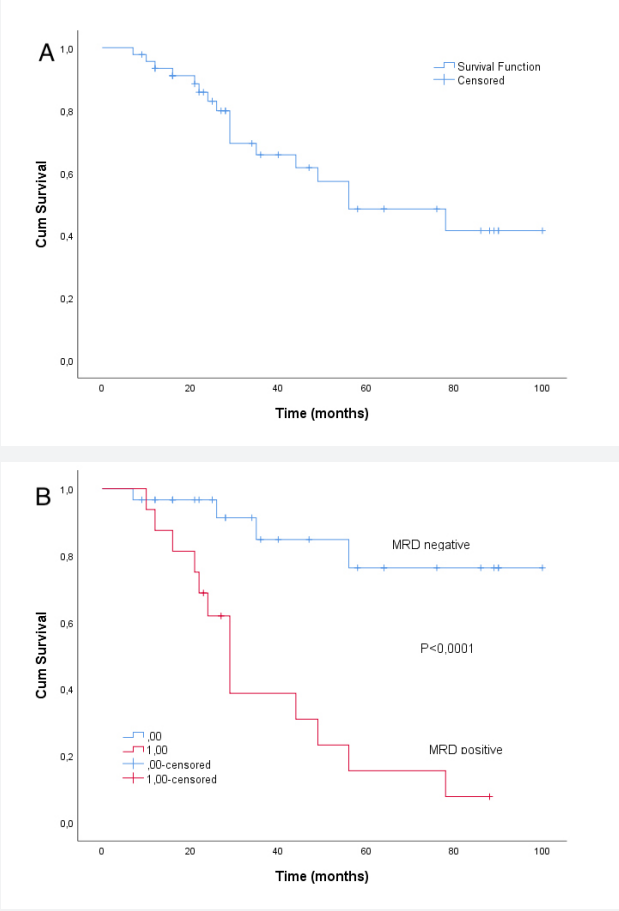

Figure 1. Progression free survival of all patients (A) and by MRD status (B)

MRD: Measurable residual disease

Most frequently used methods to measure MRD are FCM and PCR. Although HTS and more specific assays are being investigated, FCM remains the gold standard to assess MRD[11]. MRD analysis in CLL with FCM started on the basis of 2-color staining in which the classic markers CD19 and CD5 co-positivity or the unbalanced in kappa/lambda light chain ratio were evaluated, but this method did not go beyond what could be obtained by immunohistochemistry[22-24]. With the initiatives of the ERIC group and other international participants, 4, 6 and finally 8 colored FCM protocols have been developed, that remains at the moment the gold standard in prospective clinical trials[11,13]. In our study, four-color two tubes FCM

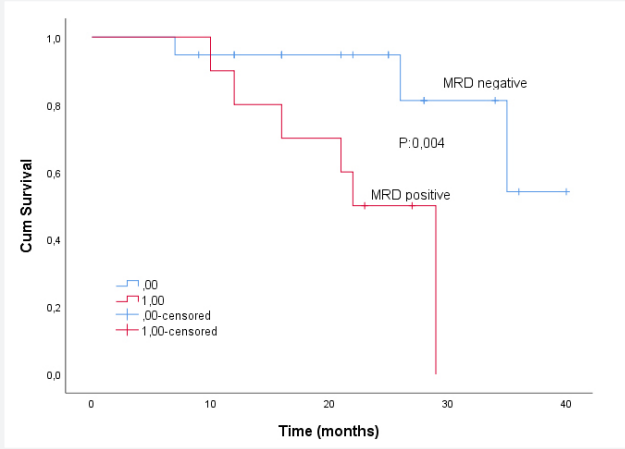

Figure 2. Progression free survival of the patients who were evaluated with dry tube method by MRD status

MRD: Measurable residual disease

Table 2. Univariate analysis on variable in relation to progression-free survival

Variable	HR (95% CI)	p value
Age	1.055 (1.003-1.109)	0.039
Creatinine	5.823 (1.068-31.73)	0.042
Albumin	5.339 (1.332-21.40)	0.018
β-2 microglobulin	1.590 (1.119-2.259)	0.01
Treatment group		
RFC	9.52	0.009
RB	4.297 (1.331-13.87)	0.015
R-CLB	9.855 (2.072-46.86)	0.004

HR: Hazard ratio, CI: Confidence interval, RFC: Rituximab, fludarabine, cyclophosphamide, RB: rituximab, bendamustine, R-CLB: Rituximab, clorambucil.
Analyses performed using univariate Cox models

method had been used until 2015, eight colors one tube FCM method was used after 2015, both of which were in line with ERIC recommendations.

Table 3. Characteristics and outcome of the patients who were analyzed by try tube method

Variable*	The patients, n=29
Age (years)	66 (45-77)
Sex, n (%)	
Male	17 (58.6)
Female	12 (41.4)
RAI stage; n, (%)	
0-2	15 (51.7)
3,4	14 (48.3)
Indication of treatment** n, %	
1	12 (41.4)
2	4 (13.8)
3	6 (20.7)
4	2 (6.9)
5	5 (17.2)
Time from diagnosis to treatment (months)	13 (1-156)
Treatment, n (%)	
RFC	9 (31)
RB	15 (51.7)
RCLB	5 (17.2)
Cycles number, n (%)	
5	2 (6.9)
6	27 (93.1)
Hemoglobin, (gr/dL)	12.15 (8.2-16.26)
Lymphocyte count, (10^9/L)	53.20 (5.4-126.2)
Platelet count, (10^9/L)	150.0 (23.0-505.0)
B2M, (mg/L)	5.7 (2.33-8.0)
Creatinine, (mg/dL)	1.01 (0.55-1.6)
Albumin, (gr/dL)	4.60 (3.0-5.23)
LDH (U/L)	254 (167-568)
CD38 positive (>%30), n (%)	1 (3.4)
Responses, n (%)	
CR	20 (69)
CRi	5 (17.2)
PR	4 (13.8)
uMRD patients, n (%)	19 (65.5)
Relapsed patients, n (%)	11 (37.9)

*Parametric variables are expressed as median (minimum-maximum)

**1: Evidence of progressive bone marrow failure 2: Massive/symptomatic or progressive splenomegaly/lymphadenomegaly 3: Progressive lymphocytosis with an increase of >50% over a 2-month period or lymphocyte doubling time of <6 months. 4: Autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids 5: Constitutional symptoms.

B2M: β-2 microglobulin, LDH: Lactate dehydrogenase, CR: Complete response, PR: Partial response, CRi: CR with incomplete bone marrow recovery, uMRD: Undetectable measurable residual disease, RFC: Rituximab, fludarabine, cyclophosphamide, RB: Rituximab, bendamustine; R-CLB: rituximab, clorambucil

In recent years, dry tube methods, which eliminate the stages such as pipetting and washing that cause cell loss and make standardization difficult, have been developed for MRD analysis[14]. Recently, Beckman Coulter has developed an eight-color tube with dried reagents which is specific for the detection of MRD in CLL samples by FCM. These tubes contain a dry antibody panel coating adhered to the bottom of tube. This tube contains ROR-α in addition to the core markers suggested by the ERIC group. In our study, this dry tube method was used in MRD analysis after March 2017. MRD was studied again with this method in the follow-up of all cases. The validity of the dry tube method in CLL samples was shown in the comparison with the method suggested by the ECIL group, but not as a clinical datum[15]. In our study, when we analyzed the patients studied with the only dry tube method separately, we observed the results similar to the total study population. Achievement of uMRD at the end of first line treatment was also significantly associated with longer PFS in patients evaluated by try tube method. With our study, clinical data of this dry tube method were presented for the first time.

Although the recently developed 8-color single-tube method reached 10^{-5} sensitivity, 10^{-4} sensitivity, which is the level proposed also by European Medicines Agency (EMA), has been used in the majority of prospective clinical studies[4-6,25-30]. To reach 10^{-5} sensitivity, at least 1 million cells must be studied. In our study, we had withdrawn an average of 500,000 cells. Therefore, in spite of that we used 8-color single-tube method, our sensitivity level was 10^{-4}.

Several large randomized controlled trials (RCT) in CLL patients have shown that MRD status after induction treatment is an independent predictor of progression-free survival and overall survival[4-6]. In 2016, after the publication of these trials, the EMA allowed the use of uMRD as an intermediate endpoint in RCTs that were used for drug approval[7] but it is still an ongoing debate whether routine MRD testing should also be a part of clinical practice. In the German CLL8 trial, MRD was analyzed in patients receiving FC or FCR treatment, with 35% of FC-treated patients achieving uMRD ($<10^{-4}$) in PB vs 63% after FCR CI. uMRD after the end of treatment was associated with significantly longer PFS than intermediate ($\geq 10^{-4}$ to $<10^{-2}$) or high MRD ($\geq 10^{-2}$)[4]. In the same study, patients who attained low-level MRD by FC chemotherapy had PFS similar to that of patients who achieved the low CLL cell levels with FCR. Therefore, authors concluded that achievement of uMRD, not the type of treatment, was the key factor for durable remissions. Similarly, in our study, low relapse rates were observed in patients who achieved uMRD irrespective of treatment regimen (RB or RFC).

In our study, uMRD was achieved in 18 (75%) of 24 patients who received FCR. The absence of patients with 17pdel and only 1

patient with 11q deletion among our patients may explain the higher rate of uMRD obtained in our study. In GCLLSG CLL10 trial, the superiority of FCR in achieving uMRD was shown compared to RB CI[19]. In our study, 12 of 17 patients (70.6%) treated with BR had uMRD. In our retrospective study, patients who received RB were unsuitable for FCR because of their age and comorbidities. Therefore, it was not appropriate to compare the two groups, although the high uMRD percentage with RB was notable in our study. None of the patients who received R-CLB had uMRD and their PFS was inferior as expected, which supports the notion that this regime is not strong enough.

In the study, the relationship between genetic changes and obtaining uMRD could not be evaluated due to the small number of patients included. IGHV mutation and ZAP 70 expression were not studied in most of the patients. CD38, which is other important prognostic factor in the studies, was not found to be related to uMRD in our study. In addition, there was no correlation with other baseline disease characteristic of the patients and obtaining uMRD.

Study Limitations

The major limitations of the study are its retrospective single-center design and limited number of patients. We were also unable to evaluate important prognostic parameters in most of our study cohort like IGHV mutation status and ZAP 70 expression.

CONCLUSION

In conclusion, MRD analysis was used in the follow-up of CLL patients outside of a clinical trial in our study and it was revealed that it could provide information about PFS in accordance with clinical studies. In addition, clinical data of dry antibody method (DuraClone RE CLB Tube) were presented for the first time.

Acknowledgement

We thank Öznür Güngör and Sinem Ülkümen for their contributions to MRD analysis.

Ethics

Ethics Committee Approval: Approval was obtained from Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (date: 29/09/2020 protocol number: 2020.219.09.06).

Informed Consent: All participants gave written informed consent for the use of clinical/laboratory data for research purposes.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.A., B.T., Concept: S.A., B.T., Design: B.T., Data Collection or Processing: S.A., Analysis or Interpretation: S.A., B.T., Literature Search: S.A., Writing: S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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